TESTOSTERONE IN THE DEVELOPMENT OF CVD IN TYPE 2 DIABETES

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INTRODUCTION

Coronary Vascular Disease (CVD) has turn out to be as the main cause for death in the realm (Detels et al.,2009) Investigators stated that by the year 2030, causality occur due to CVD will be increased worldwide. World Health Organisation (WHO) had projected that around 17 million peoples lost their life due to CVD in 2004 and approximately 20 million deaths by CVD occur globally each year, which will increases by 24 million by 2030. It has been estimated that, around 5.8 million new CVD cases every year and closely 40 million peoples with recognised CVD are currently living today (Fuster et al.,2011).

Testosterone, the chief androgen in the flow, which is produced and secreted mostly by Leydig cells (Arora et al.,2022). It effects contain vasodilatation and improvement of coronary ischemia and also possibly harmful effects.

It is generally accepted that men, come across a stable decrease in their testosterone level amidst growing age for a long time regarded as a robust risk characteristic for emerging CVD. Overall, these two particulars have determined numerous investigators to pursue for a credible association among autogenous testosterone levels and CVD (Emil et al.,2008). The fast vasodilatory effect of testosterone levels on the myocardium Ischemia in patient's misery from CVD is accredited to a thru non genomic result of testosterone on vascular cells (Kaur et al., 2021).

Hypogonadism has been reported to link with the amplified level of blood glucose, triglycerides and high body mass index, waist to hip ratio. Further, high insulin resistance are also found in hypogonadism that all the threat for increasing CVD (Jyotsna et al., 2023). Utmost epidemiological studies said that increase serum testosterone concentrations relates with reduced CVD threat in males (M Webb and Collins.,2017).

In general, testosterone disturbs the vascular wall, prostaglandin organisation, which advocates the narrowing the opening of a blood vessel and platelet accumulation

when thrombaxane development occur (Roşca et al., 2021).Developing signs designates that testosterone level play vital part in vascular health of males.

Diabetes is a common metabolic health problem, and it was estimated that over 40 million individuals diagnose with diabetes around the world. With time its occurrence is growing abnormally each year (1). Type 2 diabetes mellitus (DM) is the main form of diabetes that is related with several difficulties together with CVD (2). There is sign to designate that autogenous sex hormones illustrate key role in the development of DM (4). Some research's projected that testosterone was perhaps linked with DM risk (5). In this research, we validate that whether or not serum androgen level in men, who suffered with CVD, have lower concentration of testosterone than the normal healthy individuals.

Gap Analysis

There are several limitations of previous studies examined the association of Cardio Vascular disorder in Diabetic patients. The primary limitation of previous studies is that only few studies are available which can show the role of Testosterone in developing Cardio Vascular disorder in diabetic patients. Other limitations include the use of only self-reported diabetes status, lack of use of endocrinological testing. To our knowledge, no studies have examined the association between diabetes and CVD, using testosterone in the definition of diabetes.

Research Question

With this current proposed research, we want to explore the role of Testosterone in diabetic patients and their association in CVD.

Hypothesis

Low serum Testosterone level has been observed to strongly associate with an increased likelihood of the metabolic syndrome and has adverse clinical outcomes including CVD and premature mortality. In Diabetic patients it was observed that level of Testosterone was decreased. Additionally, there is a well-known connection between the main pathological features of Diabetes, such as blood glucose level, body fat, insulin and high risk of death from all cause and CV-related mortality. It has been hypothesized that, Testosterone likely have an impact on CVD connecting diabetes to Cardiological pathology. So, In view of this above mentioned role of Testosterone in Diabetes and CVD, we decided to explore the role of Testosterone in diabetic patients with or without CVD.

Statement of the Problem

In summary, available data favors the assumption that there is an association between testosterone and CVD. The results are however inconsistent, especially in individuals already afflicted with CVD. The dynamics of testosterone levels, prevalence of low testosterone and prognostic implications of testosterone and TD with or without CVD need further clarification. Analyses of different fractions of testosterone, i.e. total and

free testosterone which is associated with androgen receptor responsiveness are of particular interest. Such studies have the prerequisite to add a piece to the puzzle explaining this complex relationship and possibly be helpful in the search for high- risk individuals.

Null Hypothesis

- There is no relationship between type 2 diabetics' with or without CVD and socioeconomic status (SES) and their diabetes medications and physical activity adherence.
- There is no relationship between type 2 diabetics' with or without CVD self-efficacy and their diabetes medications and physical activity adherence.
- There is no relationship between type 2 diabetics' with or without CVD educational levels and their diabetes medications and physical activity adherence.

AIM AND OBJECTIVES

The aim of the study was as follows:

1. Assessment of different levels of serum Testosterone in Diabetic patients with or without CVD
2. Assessment of different biochemical parameters in Diabetic patients with or without CVD
3. Correlation of serum Testosterone with body fat percentage and fasting blood plasma glucose level

REVIEW OF LITERATURE

Various studies have been reported that low blood testosterone concentration in men with DM (Barrett-Connor et al., 1992; Ding et al.,2006; Corona et al.,2011). Opposite associations amid the blood level of testosterone and CVD risk features, such as hypertension, obesity and dyslipidemia have been witnessed (Haffner et al.,1996; Stellato et al.,2000; Tsai et al.,2004) . Further, it have been also observed that low testosterone level (LST) is intensely linked with an great possibility of the development of metabolic syndrome in men (Li et al.,2010; Liao et al.,2012; Tong et al.,2005). A study conducted on Caucasian and asian men, shows that LST is also related with hostile clinical consequences including CVD and early death (Traish et al.,2009; Ma and Tong,2010; Akishita et al.,2010). In 2010, the increased occurrence of a LST concentration in DM patients was presented in the Clinical Practice Guideline in the setting of replacement therapy of testosterone in young men with testosterone deficiency disorder. The later was demarcated by reliable signs and symptoms of androgen paucity, and an unambiguously low testosterone level in the blood. The Endocrine Society endorses analysis of pre-lunch serum total testosterone in DM patients who have signs of sexual malfunctional, impenetrable loss of weight or mobility limitation. Recently, it was studied the outcome of testosterone replacement, have generally been carried out in men with testosterone deficiency (TD) (Jones et al., 2011; Basaria et al., 2010).

Testosterone deficiency (TD) has severe clinical signs in men's health. TD influences physical and metabolic tasks, including decrease bone mineral density, decreased muscle mass and may also add to weakness. TD is linked with enhanced body fat and BMI as well as exhaustion and insulin resistance (IR). An enormous variety of CVD risk aspects is accompanying with TD. These include, metabolic syndrome, IR and DM, dyslipidemia, hypertension, arterial stiffness and atherosclerosis. Therefore, the effect of TD on CVD and associated mortality is not astonishing agreed the role of testosterone in preserving the structure and efficiency of vascular composition and possible role for growing the chance of developing vascular disease.

Till today, the reason of LST concentration in DM patients is not explained. The search of in case status of testosterone should be tested and/or swapped in prediabetic DM subjects is enduring.

Defining of Low Level of Testosterone

Defining the lower excise value of normal level of blood testosterone create a test for healthcare personals. There are certain reasons, first, the lower excise value of serum testosterone below which hostile scientific results happen in the universal population is not well-known (Bhasin et al.,2010). Second, clear differences in the standard value of testosterone value amid test centers have been stated (Boots et al.,1998). Additionally, the standard value of testosterone have been resulting before from very insignificant samples (hospital or clinic-based patients) (Bhasin et al.,2011). These methods are imperfect by their collection prejudice, as patients looking for therapeutics are further expected to have ailments than those in the overall population. So, presently, utmost population centered research uses testosterone level equivalent to the lower cut off limit, cited from (8.7-12.7 nmol/L normal range) adult caucasian male as the cut off (Rosner et al.,2007), though differences exist among interethnic population as a consequence of diverse mechanisms. Serum Free, Total and Protein- Bound Testosterone in males is produced and released into the blood circulation practically entirely by the endocrine cells of the testes (Leydig cells). It is generally bound with plasma proteins. Around 0.5–3.0% of free testosterone is total testosterone, approximately 30-40% in form of sex hormone binding globulin (SHBG) bound testosterone and 50–65% testosterone are belongs to albumin bound (Dunn et al.,1981; Vermeulen,1988). As the association of testosterone to albumin protein is nonspecific and so not tight, the amount of free and albumin tight testosterone is called as bioavailable testosterone, which imitates the hormone accessible at the cellular level (Vermeulen et al.,1999). As a substantial amount of blood circulating testosterone level is firmly attached to SHBG, changes in SHBG level might interrupt total testosterone concentration devoid of changing free or bioavailable testosterone. Situations that overpower or upsurge serum SHBG concentration without upsetting free or bioavailable testosterone level (Table 1).

Table 1: Shows the circumstances that subdue or upsurge SHBG concentration without disturbing free or bioavailable testosterone levels

1	Circumstances that subdue SHBG concentration	Obesity, DM, hypothyroidism, familial SHBG deficit, and use of steroids like glucocorticoids and androgens
2	Circumstances that enhance SHBG concentration	Aging, hepatitis, hepatic cirrhosis, hyperthyroidism, infection with HIV, and use of estrogens

Investigators tried to observe whether serum total or free androgen would be a more consistent choice when investigating the influence of testosterone. The consequences were mixed. Some described substantial relations of both serum free and total testosterone level with experimental parameters (Laaksonen et al.,2004), while others testified that merely serum free testosterone (Fukui et al.,2003) or total testosterone displayed noteworthy relations.

Research laboratory Tests of Testosterone

Total testosterone analyses are freely accessible in most test site, so, total testosterone remains the suggested initial test in the valuation of testosterone level. Serum total testosterone analysed by rapid immunoassay analyzers with substances that comprise correspondents of testosterone treated as normal reference values are providing by the makers of the analyzers. Though these immunological assays are effective and reasonable, numerous of them have imperfect printed authentication data, hovering doubt about the precision of these computerized procedures (Rosner and Vesper, 2010). Additionally, there is considerable unpredictability in the outcomes from diverse assays, typically due to the endorsement of research laboratory that are centered on the reliability of outcomes in contrast with other research laboratory by the very same analyzing kit. Some testing sites, currently assay testosterone level by liquid chromatography and mass spectrometry (LC-MS) means with improved precision than immunological assays, but are additional time-overriding and

expensive. Actually, Disease Control and Prevention Centers situated in USA (USCDCP) has started a platform since 2010 to normalize testosterone assays by means of precision-based quality control (Rosner and Vesper, 2010).

Serum Testosterone and their Epidemiology

The occurrence of low testosterone level in the blood of overall inhabitants thru diverse age clusters has been inspected in numerous significant epidemiological studies. Recently study conducted by Baltimore Longitudinal Study of Aging (BLSA) on Caucasian males, the occurrence of low total androgen amplified from roughly 20% of males with aged above 60 years, 30% males are above 70-75 years and 50% are around 80 years and above of age group. A noteworthy, self-governing and longitudinal influence of age on synthesis of testosterone has been detected with an usual alteration of (-) 0.124 nmol/L in each year in total serum testosterone (Harman et al., 2001). The same drift has been found in different part of the world such as Europe and Australia (Tajar et al.,2010; Liu et al.,2007).

A comparable tendency for men by means of long-lasting diseases has been revealed in the study group taken place in Massachusetts, in which about 415 healthy males and 1,294 males suffering with long term diseases such as cancer, CVD, DM and ulcer etc., from the America with aged group ranging with 40 to 70 years were considered. The loss of total testosterone of serum was observed about 0.4%/year in both study groups. (Feldman et al.,2002). In Caucasians, the average total testosterone level for males has been described to range 15.1-16.6 nmol/L. However, in Asians groups, increased values, ranging 18.1-19.1 nmol/L, were obsreved (Kim et al.,2012; Akishita et al.,2010). Significant topographical and ethnic changes in the values of blood sex steroids has been described. Asian males living in Hong Kong and Japan, had 20% increased total testosterone level than in Caucasians, staying in the America. Group alterations in body mass index (BMI) did not clarify the topographical variances (Orwoll et al., 2010). These variances might be enlightened by ecological and/or hereditary factors prompting the testosterone metabolism (van Houten et al., 2000).

Mechanisms of action of testosterone

Features, and masculinization, libido, and stimulation of spermatogenesis. Testosterone exercises effects generally by attaching to the subcellular androgen receptor. Diverse machineries by which testosterone move in the cell have been proposed; whom the free hormone assumed shows in Figure 1. The androgen-binding domain of the androgen receptor covers around 30% of the total receptor and is accountable for the specific attachment of androgens. Receptors of androgens are present in numerous tissues, such as testes, prostate and muscles, in addition to this also present in the kidneys and heart (Nieschlag & Nieschlag,2010). Testosterone is further transformed to other small active molecules such as estradiol through action of aromatase and dihydrotestosterone (DHT)

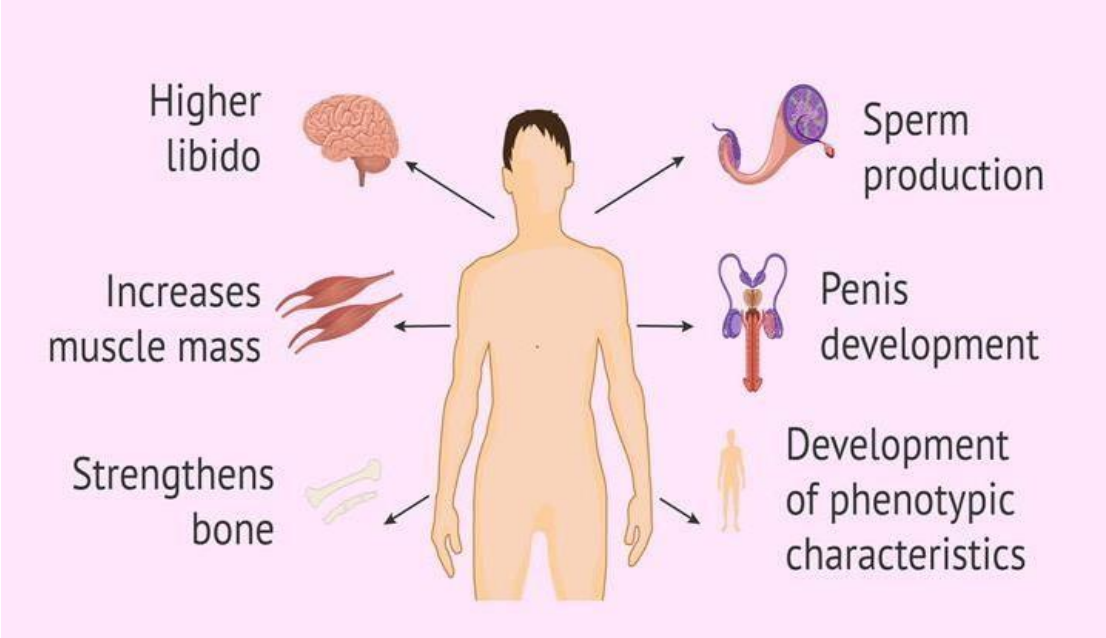


Figure 1- Different action of Testosterone

The receptor coding androgen gene for two isoforms, situated on X chromosome, has diverse handy units encompassing a DNA-binding, Trans activating and a domain that binds with hormone- respectively. A significant distinctive of the N-terminal region of the receptor contains variable repeats of CAG, coding for polyglutamine, repeats (Tirabassi et al.,2015). Population residing in Europe, the average size of the CAG tandem repeat in males is around 21-22 (Eendebak et al.,2016; Giwercman et

al.,2009). However, in common men, around 17–29 glutamine and 13–17 glycine duplications are reported. Like all the other fat-soluble organic compounds receptors, the actual interaction of the DNA binding motif of the androgen receptor encompasses two zinc fingers units. The marginal effects of testosterone are dependent mainly on an active androgen receptor, has been recommended that the amount of CAG repeat is contrariwise linked to making RNA copy from piece of DNA through androgen receptor in target tissues (Tirabassi et al.,2015). Most of the androgen receptor work done by stimulation of DNA transcription after interaction with androgen receptor, in addition non-genomic effects also observed (Michels & Hoppe,2008). In women, testosterone exerts effects either by binding to the androgen receptor or by non-genomic effects as defined for men. Testosterone play vital role in bone metabolism, gynaecological health and sexual purpose in women. Lately it was furthermore recommended that testosterone, as in men, may have role on the CVD (Spoletini et al.,2014).

Role of Testosterone on Body Configuration and Transitional Metabolic rate

There are numerous machines for the relationship of low testosterone level and DM. In various preclinical studies, knockout mice of androgen receptor established noteworthy insulin resistance quickly (Lin et al.,2005). In animal models, serum testosterone endorsed division of multipotent stem cells into muscle family, but repressed their pledge into fat cells concluded by androgen pathway. These results enlightened the famous effects of androgen therapy on body structure in men together with increase in muscle mass and decrease in fat quantity, both were likely to suppress IR (Singh et al.,2003). Additionally, effects of stem cell therapy, testosterone reduced IR by increasing catecholamine persuaded lipolysis in culture cells, and inhibiting lipase activity and triglyceride ingestion in vivo (Marin et al.,1995) . In both studies, hypertriglyceridemia suppressed insulin removal or utilization, which led the development of IR and hyperinsulinemia (Figure 2).

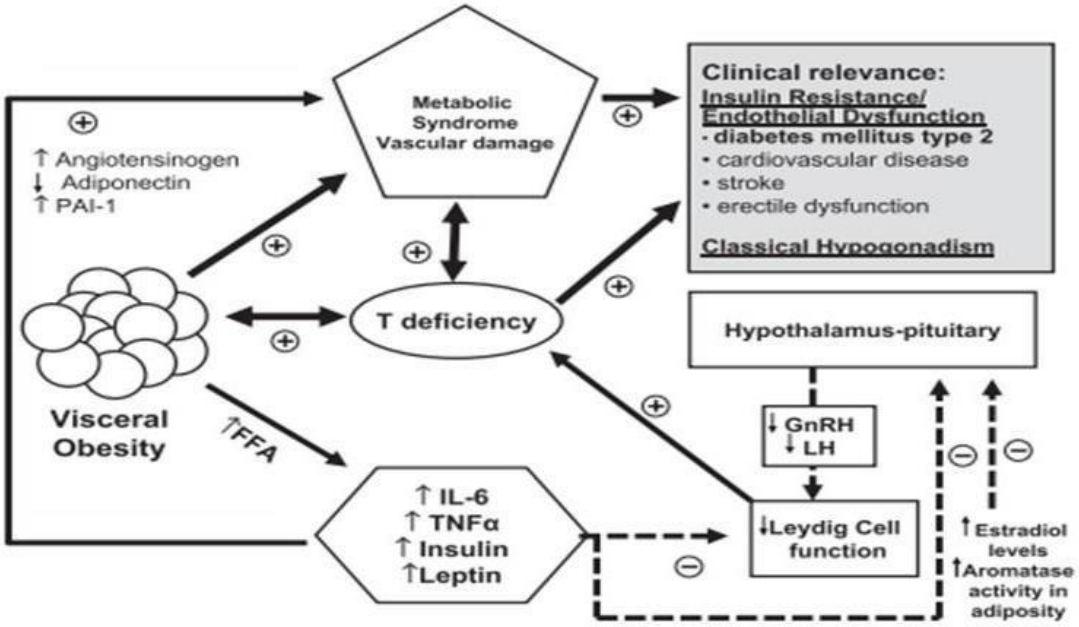


Figure 2: Complex multidirectional interaction testosterone and obesity, type 2 Diabetes (DM) leading to comorbities such as increased CVD Risk. (FFA- Free Fatty Acids, GnRH- Gonadotropin releasing hormone, LH- Luteinizing hormone, PAI-1, plasminogen activator inhibitor-1

Therefore, it has been found that by enhancing lipolysis and myogenesis, testosterone might cause the improvement in IR.

Besides, research in animal model displayed that testosterone controlled skeletal genes intricate in glucose absorption that cause the decrease in IR. Hepatic receptor of androgen signalling in the liver, repressed growth of IR in mice (Lin et al.,2008). This is steady with the autonomous and contrary link of serum testosterone level with fatty liver visible in a case control study carried out in humans (Volzke et al.,2010). Recently, a increase concentration of testosterone was found to autonomously expect a decreased possibility of DM in older people (Salminen et al.,2014), however a minor level was an autonomous jeopardy for high glucose level (Mazur et al.,2014). In the replacement therapy of Testosterone, in hypogonadal individual with either having metabolous syndrome or DM shows enhanced insulin sensitivity as analyse insulin resistance, additional supporting the signifying the IR as a facilitating aspect for the link amid low testosterone level and DM. In brief, it can be said that androgen recovers IR by altering body configuration and decreasing body fat. Though a low

blood testosterone level could add to the growth of obesity and DM through alter in body configuration. In overweight male individuals, the translation of testosterone to estrogen might decrease the bounty of LH and suppress testosterone assembly (Vermeulen et al.,1993) to aggravate the malicious series of obesity and lower blood testosterone level. Finally, it has been reported by some researchers that leptin which is expressed by Leydig cells, of testes shown to be reversely linked with blood testosterone level in men (Haffner et al.,1997), that suppress the testosterone synthesis and release, signifying their role of obesity (Caprio et al.,1999). In Caucasian men with DM, around 25–40% found to suffered with hypogonadotropic hypogonadism (Dandona and Dhindsa,2011). Further, some scientists observed that pro-inflammatory cytokines in the testes, might in the vicinity hinder testosterone synthesis in Leydig cells, and testosterone therapy in men, shown to decrease the concentration of tumor necrosis factor-α (Fukui et al.,2008). In short, various studies from preclinical to clinical trials in males all seemed to associate the probable causal part of LST level in DM and obesity, though clinical studies with large sample size are compulsory for further confirmation.

Genetic Factors of Testosterone

Genetic aspects, such as Racial and ethnic variations in lipid metabolism, could play a part in explanation the diversity of blood testosterone level and possible risk links. In an investigative study of a local Chinese population having DM and it was observed that around 10-15% of the individuals had decreased blood total testosterone level (< 2.6 ng/mL), that was inferior to the stated in white men suffering with DM (21–40%), though the alterations could be owing to ecological, conservational or civilization factors. Additional study described that only total testosterone, were lesser in younger Chinese men compare to USA men (Xu et al.,2014). In precise, it was recommended that hereditary aspects, such as ethnic alterations in sex hormone metabolism, could play vital role. In Asians, genetic mutation of uridine diphosphate-glucuronosyltransferase (UGT2B17) was related with suppressed androgen glucuronidation, causing in increased dynamic androgen level in Asians as matched to white men, as in later ethnic groups glucuronidated of androgen led the inactivation of its faster. As compared to Caucasians, in Asian individuals the occurrence of this

deletion polymorphism in UGT2B17 was around 22 time higher (Swanson et al.,2007). Other investigators have recommended that ecological, but not hereditary, issues affects serum total testosterone. Here, Chinese men living in the USA had increased level of total testosterone than Chinese men staying in china, but similar levels by Caucasians residing in the USA. This kind of variations as observed within the identical ethnic group has been accredited to environmental and ecological impacts (Wang et al.,2007). In present time of worldwide proliferation, the chemistry between hereditary and ecological factors, and their links with blood testosterone concentration warrants additional study, which will pitch vision into the cause of disease, analysis and controlling of hypogonadism in diverse area of the biosphere for patients with diverse races and civilizations.

Androgen receptor (AR) gene polymorphism are key to determine testosterone effects (Zitzmann et al.,2001). Its stimulus induced activity was negatively correlated with length of CAG repeat chain (Francomano et al.,2013). Noteworthy traditional variances had been detected in the size of the CAG chain. The size for Asians is around 22–23, white men 21–22, and Africans black were 18– 20 neucleotide separately (Francomano et al.,2013). Further, study on European men shows an enhance level of estrogen/androgen ratio in connotation with lengthier CAG repeat for AR (Zitzmann et al.,2003). Though, the sizes of CAG chain also different amid persons of the identical ethnic cluster. As observed in Indian males having with size of CAG repeat ≤19 had higher risk of developing prostate cancer (Hakimi et al.,1997). So, measuring the mutation at AR level might be a possible device en route for customized valuation and action of hypogonadism.

Aging effect on Testosterone Level

Aging is very well known and documented view that shows in a deterioration in endocrine sex hormone concentration, and is possible a mixture of testicular and pituitary with hypothalamic flaws. In aging males, there was condensed testicular reaction to gonadotropins with repressed and changed tremor of the hypothalamic beat producer (Tajar et al.,2012). In numerous large population size study, a substantial consequence of age on total serum testosterone concentration had been

perceived (Tajar et al.,2012). A substantial opposite suggestion between serum testosterone and insulin levels free from age has also been described in Caucasian men (Simon et al.,1992).

Testosterone and the metabolic syndrome (MES)

Low testosterone is also shown to usually related with a great occurrence of MES (Brand et al.,2011; Corona et al.,2011). MES is a bunch of metabolic risk features with IR, dyslipidemia, hypertension and obesity, in addition aspiring by transformation and altering lifestyle. There is robust epidemiological statistics supporting the prognostic assessment of MES for additional growth of DM/CVD, though there is constant debate going on whether, this is by way of consequence of preservative or multiplicative influence of these constituents. More existing data proposed connection of this disorder with other ailments including non-alcoholic fatty liver disease (Hamaguchi et al.,2005), long lasting kidney disease (Jindal et al.,2012), arthritis (da Cunha et al.,2012) and even cancer (Chen et al.,2012). In a research of male having low testosterone level with MES and DM, testosterone therapy decreased blood pressure more efficiently than diet regime and workout alone (Heufelderet al.,2009). In patients, who taken androgen deficiency rehabilitation with gonadotrophin-liberating endocrine release-protagonists for prostate cancer, there was thickening of large blood vessel linked with a severe reduction in blood testosterone level (Smith et al.,2001). Further, it was reported that testosterone has involve in expanding of blood vessels in both human and animals, signifying that it would be an vital controller of vasculature defiance and transformer of blood pressure (Webb et al.,1999).

In the Telecom study, Scientist documented that LST was correlated with enhance concentration of bad cholesterol. A related lipid profile had been testified in males with intensely LST level secondary to prostate cancer management (Haidar et al.,2007). Heufelder et al. first and foremost stated the positive effects of testosterone therapy on lipid profile in individuals having MES and DM. Though, most works reported that alterations in testosterone level cause to variations in body configuration, IR and the occurrence of MES, the contrary would be also be occur. Other potential

studies have observed that growth of MES enhanced the advanced age related deterioration in testosterone level (Haring et al.,2010). Men with DM, any alteration in blood testosterone level with time associated contrariwise with changes in IR, hovering the likelihood that routine and pharmacological therapy of DM that recover IR might also pay the way to augmented blood testosterone level.

Sexual dysfunction and low testosterone level in DM

Depression and ED are the prime reason in males, due to which sexual desire is decreased. Furthermore, Study in European Male Aging, low testosterone level in serum was more common in males with co-occurrence of disorders such as metabolic syndrome, obesity, and DM. Data obtained from diabetes clinics shows that testosterone levels were found to decrease in men with DM (Kapoor et al.,2007) as compared to testosterone levels obtained in males with and without ED and DM and it was concluded that lower the testosterone level of serum in males, higher the severity of ED (Kapoor et al.,2007a). Though enrichment in glucose level is accompanying with some perfection in erectile function in some research, majority of clinicians have not observed this to be a consistent and actual therapy for ED. In addition, Testosterone therapy enhanced libido (Jones et al.,2011) by improving the numeral of night-time erections, sexual feelings and sum of fruitful intercourse meetings, and total sexual pleasure in men with cut off value of serum testosterone is 346 ng/dL (,12 nmol/L). The influence of testosterone on the desire for sex was more reliable than on erectile function.

Association between low testosterone level and CVD risks,

Increasing results obtained from multiple studies shows that low serum testosterone (LST) is has great importance and association which increases in all-cause death, in addition to the metabolic syndrome and DM (Shores et al.,2006; Laughlin et al.,2008; Haring et al.,2010). Now it is well documented that LST value is used to predict the bigger chances of developing of CVD independent of obesity, age, or lifestyle in males with or without DM (Khaw et al.,2007; Laughlin et al.,2008; et al.,2010). Further it was confirmed by the study on patients with CVD, shows excess mortality in the testosterone lacking males compared with healthy males having normal serum

testosterone levels (Malkin et al.,2010). In addition, testosterone insufficiency and CVD both are linked with abnormal lipid metabolism result in metabolic syndrome, DM, hyperlipidemia, and aberrations of coagulation (Maggio and Basaria,2009). In case control studies on a small size of subjects, testosterone dosing caused dilation of coronary artery, reduced myocardial ischemia during stress tests (English et al.,2000).

The decrease in circulating testosterone level that happens as men become old as compared to healthy young males is a predictable occurrence (Handelsman et al.,2015; Travison et al.,2017). Further, these changes may also relat to the occurrence of obesity and other health comorbidities (Shi et al.,2013). Thus, there is a substantial attention in whether condensed flowing level of androgens are threat interpreters for increase occurrence of CVD, and if occur, then an opportunity to discover therapeutic intervention (Yeap et al.,2012). Immunological assays are mostly used for the assessment of sex steroid values, but there is chances of developing errors, as assays are not full proof of contaminations, can under or overestimate certain hormone levels when equated with the usual method such as mass spectrometry. For longitudinal studies of this type, in which same individuals are examines repeatedly over period of time to detect an link is related to the amount of outcome (Schoenfeld,1983), thus studies have examined greater numbers CVD-related events or deaths are expected to be more useful and are characterized as a complex consequence of CVD events, precise outcomes of incident stroke or deaths due to CVD.

MATERIALS AND METHODS

Setting: A total of 150 subjects with (50 from each group; Group A – Normal Healthy Control, Group B - DM subjects without CVD, Group C - DM subjects with CVD) age and BMI matched males of 30–70 years age group were recruited attending the OPD of General Medicine of Medical/Dental college, Santosh Deemed to be University Ghaziabad (UP), India, to fulfil the inclusion criteria were enlisted for the study.

Duration of study: 12 months (December 2022-December 2023)

Inclusion criteria: Patients with DM with or without CVD of 30–70 years were recruited and gave written voluntary consent were recruited for the study.

Informed consent form was accompanied by a full remedial history and medical investigations were taken of all the enlisted subjects.

The study subjects who participated in this study was described each and everything of the study and a written approval was acquired.

Exclusion Criteria

Subjects affected from cardiac incident (myocardial infarction, uneven angina, myocardial angioplasty) previously. Further, persons suffering from disorders like liver disease, hypothyroidism, cancer etc and sign of any recurring infection like osteomyelitis and ulcers or any known antiquity of hypogonadism were excluded from the study.

Ethical approval

The study had the approval of the medical and ethical committee for research committee of Medical/Dental college, Santosh Deemed to be University Ghaziabad (UP), India (F.No. SU/R/2024/1344[3]). All the examinations were carried out as per the Helsinki and Tokyo Declaration. A total of 150 subjects with (50 from each group; Group A – Normal Healthy Control, Group B - DM subjects without CVD,

Group C - DM subjects with CVD) of 30–70 years were recruited and gave written voluntary consent were recruited for the study and divided in three different groups-

Chemical and reagents

Commercially accessible kits were purchased for assessment of biochemical variables such as fasting blood glucose (FBG), High sensitive C-reactive protein (hsCRP). Serum Total Testosterone (TT), Free Testosterone (FT), bioavailability of testosterone, Luteinizing hormone (LH), insulin, and Follicle-stimulating hormone (FSH) were examined using radioimmunoassay (RIA) kits (Beckman Coulter Inc.). Further, Glycated hemoglobin (HbA1c) was analysd by using HPLC (Bio-Rad D10).

Assessment of Body Mass Index

Body Mass Index (BMI) will be calculated from the measured height and weight by using Quetelet's Index (Nuttall,2015). BMI is measured by dividing weight in kilogram by height in meter square. The procedure was explained to all the people who participated in this study.

Measurements of Body fat percent

Body fat percent will be calculated by applying the four skinfold measurements into the Durnin and Womersley formula (Davidson et al.,2011).

Assay of fasting blood glucose (FBG)

Plasma glucose was estimated by using GOD-POD method from commercially available kit (fortress Diagnostics Limited, United Kingdom).

Principle

It is an enzymatic test which is based on the formation of a pink quinoneimine dye. In this reaction plasma glucose reacts with the enzyme glucose oxidase and forms Gluconic acid. The hydrogen peroxide formed is catalyzed by peroxidase and reacts with phenol and 4-aminoantipyrine to form the dye indicator. This dye has maximum absorbance at 505 nm. The intensity of the color complex is directly proportional to the concentration of glucose in specimen.

GOD

Plasma Glucose + O2 + H2O $\longrightarrow$ Gluconic Acid + H2O2 POD

H2O2 + 4-aminoantipyrine + Phenol $\longrightarrow$ Quinoneimine (Red dye) + 4H2O

Procedure: 1 ml of enzyme reagent was mixed with 10 µl of test serum/standard and incubated for 15 min at 37 °C or 30 min at room temperature. After incubation, the absorbance of the obtained product was measured at 505 nm against reagent blank. The final color was stable for two hours.

Calculation:

Glucose in mg %= Absorbance of test sample / Absorbance of standard x 100.

Assay of High sensitive C-reactive protein (hsCRP)

High sensitive C-reactive protein (hsCRP), was assessed by standard ELISA kit as per manufacturers instruction (Chondrex, Inc., Woodinville, USA) using automated plate reader (Thermo Scientific, USA).

Principle of ELISA

It is a common plate (usually a polystyrene multiwell plate) based laboratory technique designed for detecting and quantitative analysis of specific analyte usually an antigen/antibody, in a liquid sample or wet sample which result in a colored end product that correlates with the amount of analyte present in the sample. All ELISAs rely on the specific interaction between an epitope, a small linear or three dimensional sequence of amino acids found on an antigen, and a matching antibody binding site.

Procedure

Briefly, in ELISA, a liquid sample with an unknown amount of antigen is added to a polystyrene microtiter plate. This is followed by addition of conjugated antibody with an enzyme (horseradish peroxidase). This enzymes catalyse the hydrolysis of a chromogenic substrate, such as 3,3',5,5'-tetramethylbenzidine (TMB), which undergoes a colorimetric change (e.g. color development) due to the formation of end product. The quantity of the analyte is measured by using a spectrophotometric plate

reader at particular wavelength Figure 3..

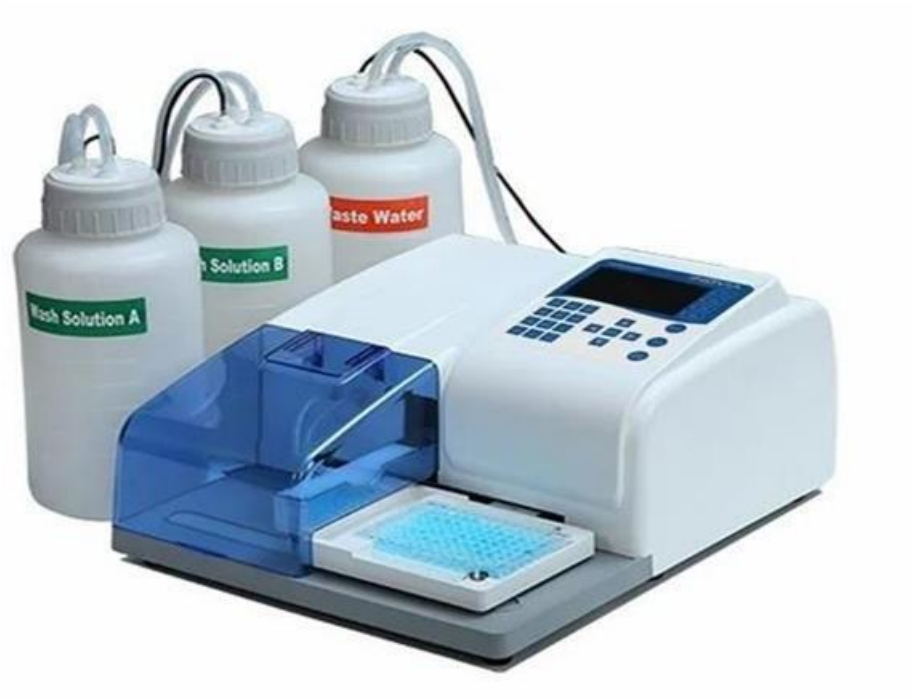


Fig. 3: ELISA Reader

Assessment of Testosterone and bioavailability of testosterone

Serum Testosterone were measured using radioimmunoassay kits (Immunotech, Beckman Coulter Inc., Czech Republic).

Principle

The radioimmunoassay of testosterone is a competition assay. Samples and calibrators are incubated with 125 I-labeled testosterone, as a tracer, in polyclonal antibody- coated tubes. After incubation, the contents of the tubes are rinsed so as to remove unbound 125 I-labeled tracer. The bound radioactivity is then determined in a gamma counter. The testosterone concentrations in the samples are obtained by interpolation from the standard curve. The concentration of testosterone in the samples is indirectly proportional to the radioactivity.

Procedure

Step 1 (Additions)

To coated tubes, add successively 50 µL of calibrator, control or sample and 300 µL of tracer. Vortex gently 1-2 seconds.

Step 2 (Incubation)

Incubate 1 hour at 18-25°C with shaking (≥ 400 rpm).

Step 3 (Counting)

Aspirate carefully the content of tubes (except the 2 tubes «total cpm»). Wash once with 2 mL of wash solution. Count bound cpm (B) and total cpm (T) for 1 minute.

(*Add 300 µL of tracer to 2 additional tubes to obtain "total cpm".)

Calculation:

Calculations were carried out by using standard curve.

Assessment of Free Testosterone

Serum Free Testosterone were measured using radioimmunoassay kits (Immunotech, Beckman Coulter Inc., Czech Republic).

Principle

The radioimmunoassay of free testosterone is a competitive assay. The procedure follows the basic principle of radioimmunoassay where there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites . The amount of [125 I]-labeled testosterone analog bound to the antibody is inversely proportional to the concentration of unlabeled free testosterone present. The separation of the free and bound antigen is achieved by decanting or aspirating the antibody-coated tubes. A standard curve is constructed and unknown free testosterone values are obtained from the curve by interpolation.

Procedure

Step 1 (Additions)

To coated tubes, 50 µL of calibrator, control or sample, immediately add 200µL of

tracer. Mix by vortex.

Step 2 (Incubation)

Cover and incubate 60 min in a water bath at 37 ± 2°C. Decant all tubes, (except «total cpm» tubes), by simultaneous inversion with a sponge rack into a radioactive waste receptacle. Strike the tubes sharply on absorbent material to facilitate complete drainage. Add 3.0 mL of deionized water to all tubes, except «total cpm» tubes.

Step 3 (Counting)

Decant all tubes, (except «total cpm» tubes), by simultaneous inversion with a sponge rack into a radioactive waste receptacle. Strike the tubes sharply on absorbent material to facilitate complete drainage. Drain on absorbent material for a minimum of 2 minutes. Blot droplets from the rims of tubes. Count bound cpm (B) and total cpm (T) for 1 minute (*Add 200 µL of tracer to 2 additional tubes to obtain "total cpm".)

Calculation:

Calculations were carried out by using standard curve.

Assessment of LH, Insulin, and FSH

Serum LH, insulin, and FSH were examined using radioimmunoassay (RIA) kits (Beckman Coulter Inc.) as per manufacturer's instruction Figure 4.

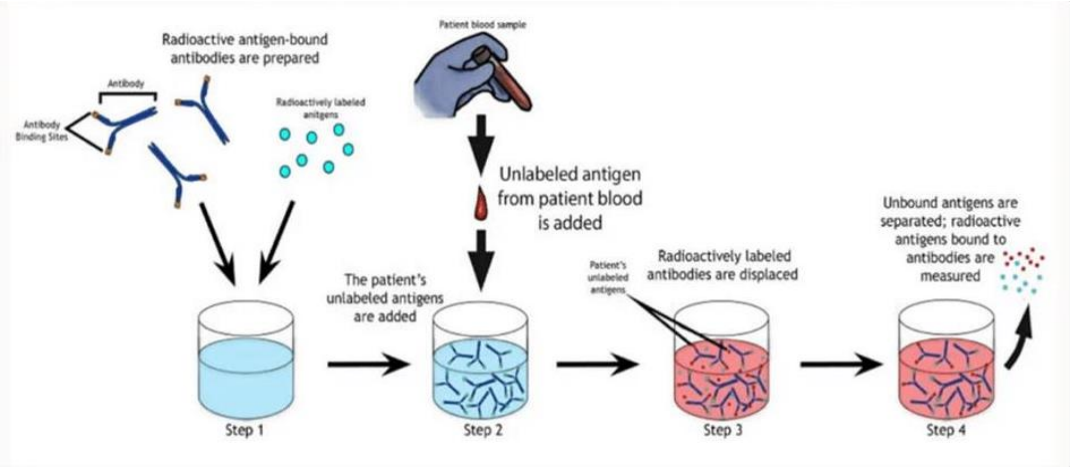


Fig. 4: General procedure of RIA

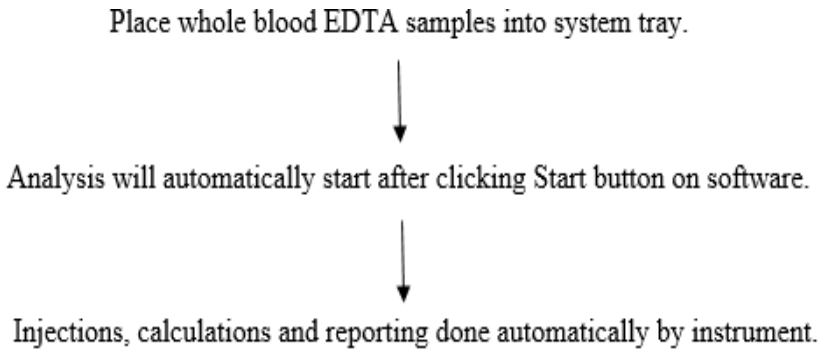
Assessment of Glycated haemoglobin (HbA1c)

Glycated haemoglobin was analysed by using HPLC as per manufacturer's instruction (Zivak Technologies, corner BMA, Quezon Ave., Quezon City.

Principle

The whole blood specimen is diluted with hemolysis solution. Then hemolyzed samples are injected to a cation exchange column. HbA1c is separated from other species by using a pH gradient and ionic interactions between cation exchange group on the column material and the hemoglobin components Figure 5.

Procedure


Place whole blood EDTA samples into system tray.
Analysis will automatically start after clicking Start button on software.
Injections, calculations and reporting done automatically by instrument.

Prepared samples are stable for 24 hours at 2-4 °C temperature.

Fig. 5: Ion-Exchange HPLC System: D-10

Statistical analysis

We used One-way ANOVA followed by post hoc Tukey's test for comparison categorical variables between quantitative variables. Further, for correlation study Pearson's coefficient of correlation was applied. All data were shown as Mean ±SD. Data were reflected significantly different if $p < 0.05$.

RESULTS

In this study we enrolled 50 healthy subjects and 100 patients (50; DM without CVD and 50; DM+ CVD) and there anthropometric features of cases and controls are presented in Table 1. Mean age were 56.22±7.68, 57.72±7.51 years respectively, and the mean body mass index (kg/m2) were25.27±3.52, 24.90±3.35 are presented in Table 1. The median Fat mass was 16.74±6.40 16.46±6.32. No Noteworthy alterations were witnessed between BMI, Fat mass, and fat body percent when cases were equated with the healthy control group Table 2.

Table 2: Systematic assess of the physical features (Anthropometric) presented in the three different study groups

Anthropometric parameters	Control	DM	DM+CVD	P Value
Age (years)	57.08 ± 7.80	56.22 ± 7.68	57.72 ± 7.51	NS
BMI (kg/m2)	24.57 ± 3.66	25.27 ± 3.52	24.90 ± 3.35	NS
Fat mass	14.75 ± 6.68	16.74 ± 6.40	16.46 ± 6.32	NS
Calculated body fat percent	28.21 ± 2.75	29.35 ± 1.25	29.34 ± 4.40	NS

BMI: Body mass index

NS: Non significant

Table 3: Shows the baseline characteristics between the cases and healthy individuals of HbA1c, serum insulin, LH and FSH.

Biochemical parameters	Control	DM	DM+CVD	P Value
HbA1c (%)	5.54 ± 0.31	8.08 ± 1.62	8.32 ± 1.41	a<0.001 b<0.001 c=NS
Insulin (µIU/ml)	5.01 ± 1.05	5.51 ± 2.35	6.65 ± 4.21	a= NS b= NS c= NS
LH (IU/L)	6.12 ± 1.08	7.12 ± 1.41	6.08 ± 2.51	a= NS b= NS c= NS
FSH (IU/L)	11.21 ± 3.21	9.05 ± 1.01	9.21 ± 1.41	a= NS b= NS c= NS

a: Groups A versus Groups B, b: Groups A versus Groups C, c: Groups B versus Groups C

Table 3 displays the baseline features between the cases (DM without CVD and DM+ CVD) and healthy control individuals; Significant ($p < 0.0001$). The average value of HbA1c, and serum insulin were 8.38±1.93, 8.60±1.75%, and 6.38±6.40 7.73±7.73 µIU/mL, respectively. Further level of hormone LH, FSH were also observed and we found their mean levels are 7.63±3.88, 6.28±3.03 and 9.41±4.91 9.50±5.51 IU/L respectively.

Level of FBG and hsCRP in study subjects

The mean level of FBG and hsCRP was determined, and we found that FBG and hsCRP leavels were higher than health control groups and was statistically significant (Figure 6).

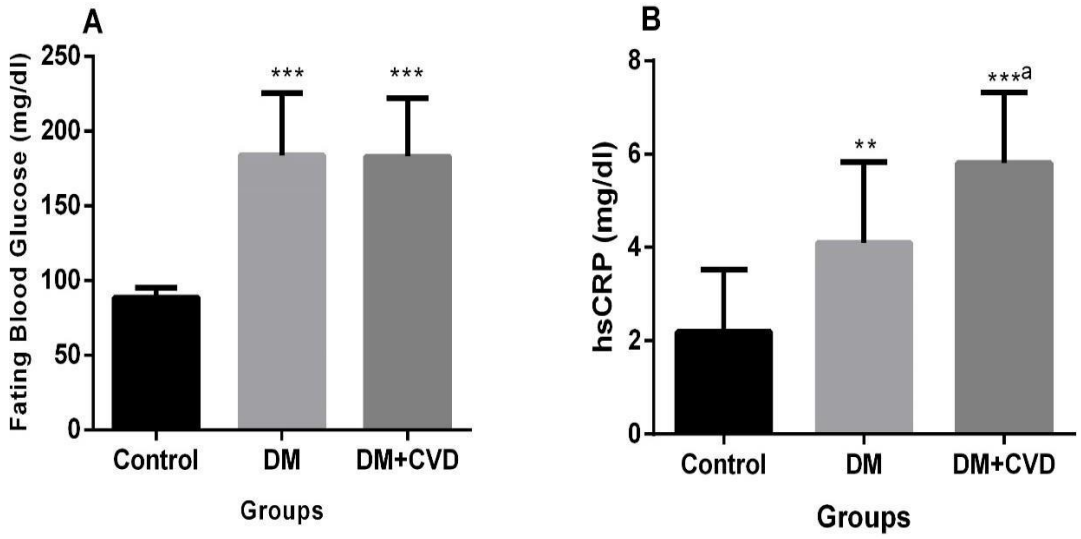
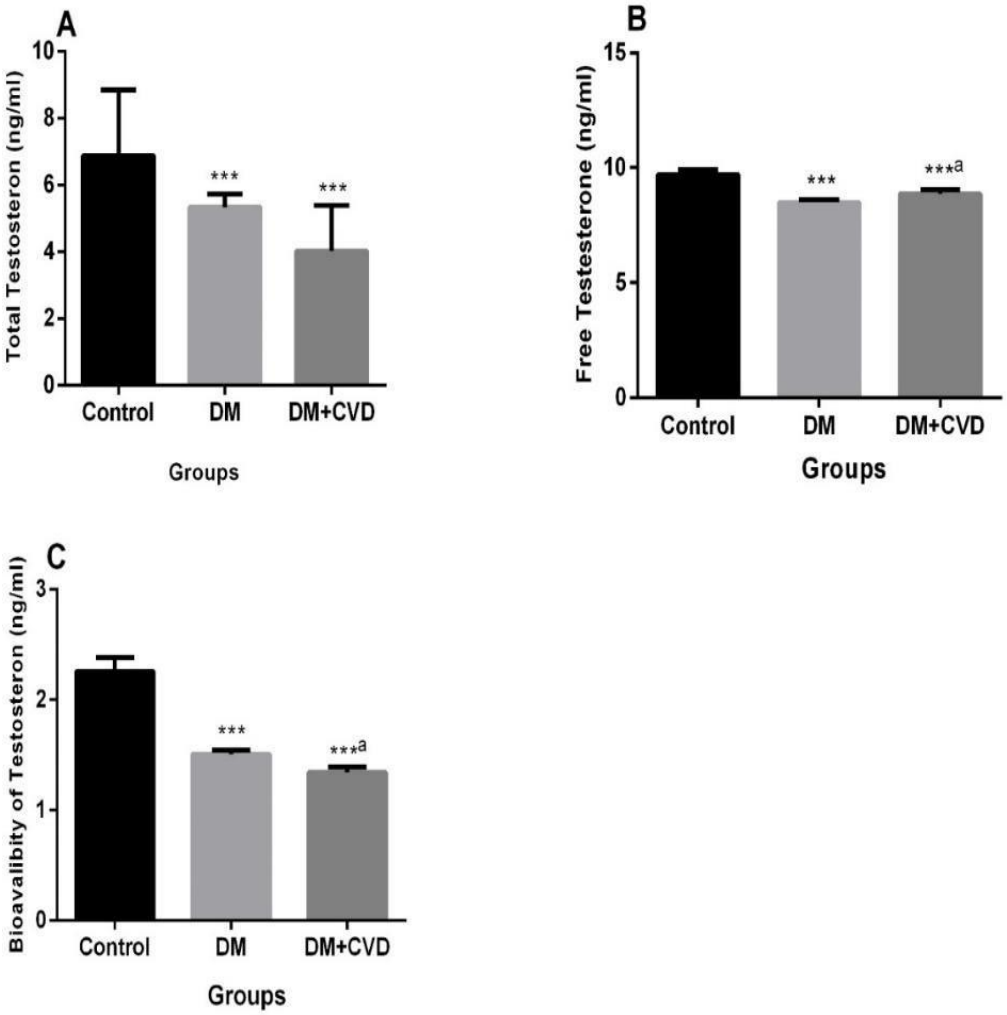


Fig. 6 : Shows the bar diagrams of the fasting Blood glucose (FBG), and hsCRP

Level of Testosterone, CVD and Diabetes

The level of total testosterone, free testosterone and bioavailability of testosterone were determined, which was lesser than that in the healthy control group and was arithmetical significant. There is cumulative sign from several studies after amendment of confounding variables that LST is linked with a rise in all-cause death that is free from metabolic syndrome and diabetes. LST also expected the amplified risk of CVD that was free from hyperlipidemia, obesity, age and living standard in males with or without DM. It was observed that in patients suffering with CVD excess death was found in the testosterone-deficient males equated with males with usual serum testosterone levels (Figure 7).



Figures 7: Shows the bar diagrams of the level of total testosterone, free testosterone and bioavailability of testosterone.

Correlation study

The Pearson correlation coefficient showed a tendency toward a positive correlation with total testosterone and body fat percent. However, this positive correlation was not statistically significant for free testosterone (Figure 7A). A correlation between the bioavaibility of testosterone level with body fat percent was also not statistically significant, nor was the correlation between the BAT and the BF (Figure 7B). However, the total testosterone level was not significantly correlated with the FBS (Figure 7C).

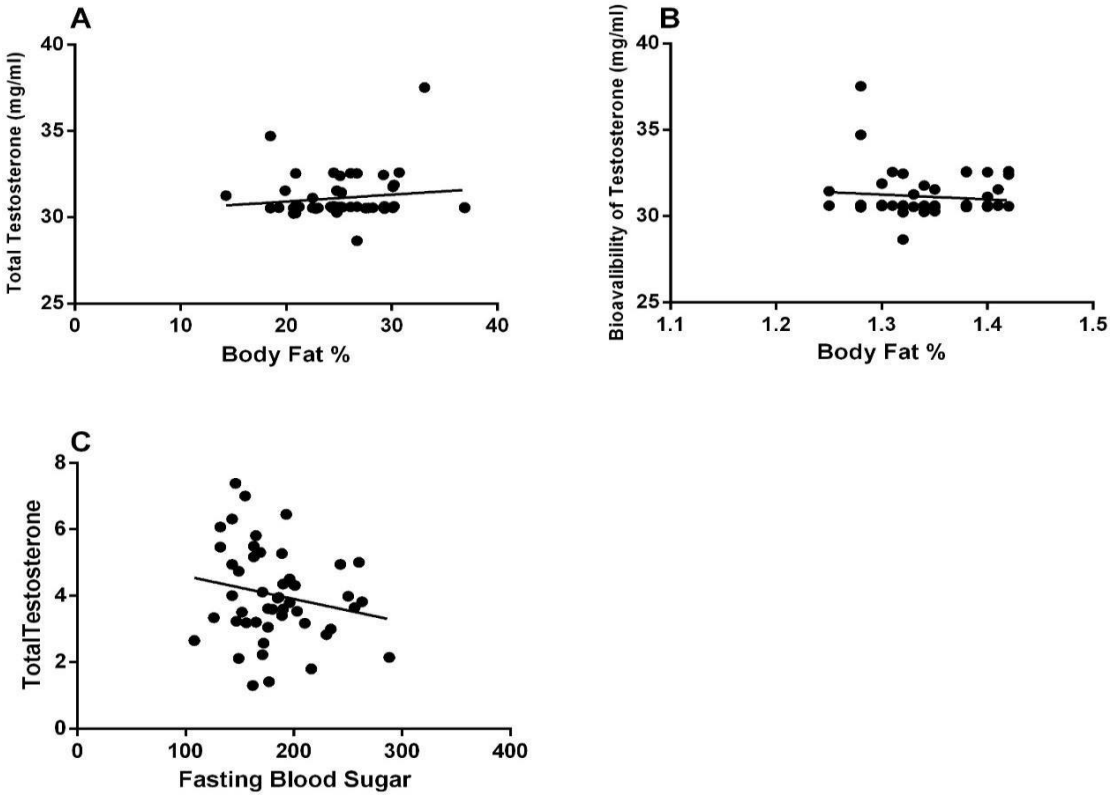


Figure 8: Representation of the correlation analysis. (8A). There was a trend toward a positive correlation between the BF and the total T level. (8B).There was a trend toward a negative correlation between the BAT and the BF (8C) Negative correlation between the Total T level and FBS.

DISCUSSION

Diabetes is a developing epidemic with millions of people suffering globally and this figure is likely to increase exponentially by 2035 (Danaei et al.,2011). Occurring of Cardiovascular proceedings is the most vital cause of death in individuals suffering with diabetes. Recent epidemiological studies shows that there is clear association between dysglycemia and obesity along with metabolic disorders with LST in diabetes ((Haring et al.,2011; Schipf et al.,2011; Svartberg et al.,2006)). This connotation is of medical implication as low testosterone in males has been found to be linked with increased CVD risk factors, including dyslipidemia and atherosclerosis and generally escalation in death (Araujo et al.,2007). Testosterone is the key androgen in the male reproductive system that is accountable for the progress of secondary sexual characteristics such as deepens of voice, growth of facial and pubic hair, sexual need, and erection. It is produced in the testis and release into the blood circulation, where it applies their various effects only after binding with androgen receptor. To withstand the appropriate levels of testosterone, a pulsating system of many interconnecting factors implicated relating the androgen targeted organs has to be in perfect balance (Jameson et al.,2010). There is significant support regarding level of testosterone in elderly men through MS and CVD which has exclusively altered its vision (Jones et al.,2007).

Insulin resistance, one of the hall marks of diabetes among males was found to be related with LST levels. Previous it was well reported that IR was accompanying with LST levels in males since testosterone regulates the synthesis of glycogen in muscle (Holmang et al.,1992; Holmang and Bjorntorp,1992). LST levels, including both blood free and total testosterone, were firm to be linked with amplified death among elderly men (Shores et al.,2006). It can be recommended that glycaemic control may control testosterone concentration by either influencing synthesis of glycogen or hypogonadism amid diabetic maes, this procedure is associated to kisspeptin (a neuropeptide), which has been witnessed to be downfall in increase glucose levels in numerous preclinical studies (Castellano et al.,2006). In fact, treating of kisspeptin

among males in which synthesis and secretion of sex hormones are very low, augmented the release of LH, which also amplified internal testosterone release. These research's narrate hypogonadism and diabetes (Grossmann et al.,2008; Kapoor et al.,2007). Heufelder and cols (Heufelder et al.,2009), have suggested the useful consequences of testosterone therapy on diabetic patients. Though, few studies have revealed contrary outcomes, i.e., positive association between total testosterone levels and HbA1c but no association with FBG (Corrales et al.,2004).

In this study, we likened different form testosterone levels of DM with and without CVD with healthy controls. For this, we analysed the TT, FT, and bioavailable testosterone in every study participants which permitted an inclusive understanding of testosterone status in DM with CVD. We establish the uppermost occurrence of hypogonadism as specified by LST in DM subjects with CVD followed by DM subjects without CVD. However, in normal healthy control level of testosterone is normal. We found hypogonadism in the most of the subjects with deficiency in testosterone level. Our results in accordance with previously published data which also showed that diabetic men had considerably lower LST levels when equated with normal healthy men. They also establish that men with advanced LST levels had a 42% lesser threat of having DM than those subjects who belongs with lower levels of testosterone. Further, in another case control study performed on 1,292 men, (Brand et al.,2011), shows that DM men had both lower testosterone concentration and sex hormone binding globulin (SHBG) as compared with healthy men. Testosterone deficit was observed in men with diabetes, irrespective of kind of diabetes. Recently study conducted on 1,849 men containing 1,451 healthy and 398 DM, displayed that testosterone levels were also affect by the obesity, as they reported a negative link amid testosterone level and BMI, regardless of subjects having diabetes or not, though DM men had a advanced occurrence of LST across all BMI categories (Mulligan et al.,2006).

Further we also assess body fat, to see whether, lack of serum testosterone concentration was linked to its BMI or fat content. It has been assumed that amplified content of body fat tributary to deficiency of testosterone levels hints to improved change of testosterone to estradiol (Cohen,1999). This led the improved destruction of

hypothalamic GnRh (Hayes et al.,2001; Pitteloud et al.,2008) and LH (Jones,2007) and consequent hypogonadism. Main hypogonadism which has been described less frequently is supposed to happen secondary due to vascular injury in the testis (Petak et al.,2002; Seftel et al.,2006). Testosterone expressions negatively correlated with the body fat percent. However, TT levels was positively correlated with body fat percent. These influences of testosterone insufficiency may be causative considerably to the related risk of CVD in DM men. It has been assumed that LST endorse amplified deposition of fat and growth in adiposity (Cohen,1999). Previously, cumulative studies have discovered that men sex hormones paucity is linked with threat for CVD (Morris and Channer ,2012; Zhao and Li,1998) in addition its influence to body muscle mass and adiposity. It has been stated that LST are connected with arteriosclerosis indicators such as amplified CIMT (Vermeulen et al.,1999; Svartberg et al.,2006). Substantial testosterone deficit witnessed in diabetes individuals with CVD increases the vibrant question like these individuals should go through replacement therapy of testosterone in an effort to decrease development of CVD risk. This concept is also centered on the outcomes of a latest retroactive study including around 83,000 elders (Sharma et al.,2015). Number of previous studies shows that stabilization of TT intensities with replacement therapy for testosterone that led to a noteworthy depressing of all reasons of death (Sharma et al.,2015). Further, additional works have too testified that amended glycemic control, condensed IR and amplified insulin sensitivity in DM getting testosterone replacement therapy (Kapoor et al.,2006; Kalinchenko et al.,2010), which then lead to summary CVD risk in DM. Additional, it has been establish that testosterone replacement therapy decreases fattiness and visceral mass and ratio between waist and hip (Kapoor et al.,2006). Result of testosterone replacement on insulin sensitivity is believed to be mediated by androgen receptor. As this are present in abundant quantity in visceral fat patches than hypodermic fat depots (Joyner et al.,2002). The consequences of the current study established that, subjects suffering with CVD have diminished levels of TT (Zhao and Li,1998). The study of our results are in consistent with the present study. Our study also divulges that BMI of CVD patients are increased than the healthy normal individuals, but statistically not significant.

Limitation

This study was conducted in single hospital, and we draw solitary point sampling and not recurrent sampling was carried out. So, procurement of multipoint sample could have providing more precise valuation of LST levels in males and it relics a constraint of the current study. Thus, additional studies are essential with a larger sample size to establish the role of testosterone on the DM with or without CVD.

CONCLUSION

CONCLUSION AND FUTURE PROSPECT

In summary, our study illustrates how LST in diabetes contribute to the divergent trends in CVD and mortality in India. The complications of diabetes often lead to the death of most diabetic patients, since the risk for stroke and heart disease is much higher when compared to adults without diabetes. The co-existence of these two diseases in a society with an increasing mean age is a significant issue. The underlying biological mechanisms that link the development of CVD in diabetes are not fully understood.

In the current study we try to explore the role testosterone in diabetic mens in connection with CVD. We observed that level testosterone was significantly low in the diabetic subjects suffering with CVD as compared with healthy subjects and diabetic subjects without having any complications relate to CVD. This was further correlated positively with FBS and fat percent. Testosterone deficiency (TD) has severe clinical signs in men's health and influences physical and metabolic tasks, including decrease bone mineral density, decreased muscle mass and may also add to weakness. TD is linked with enhanced body fat and BMI as well as exhaustion and insulin resistance (IR). An enormous variety of CVD risk aspects is accompanying with TD. Therefore, the effect of TD on CVD and associated mortality is not astonishing, agreed the role of testosterone in preserving the structure and efficiency of vascular composition and possible role for growing the chance of developing vascular disease.

This study recommends that LST can significantly increases the chances of CVD among men suffering with DM. Differences in the LST can be castoff as a biomarker to evaluate the development of DM. In accumulation, testosterone replacement should be reflected to better clinical outcomes amid DM men.

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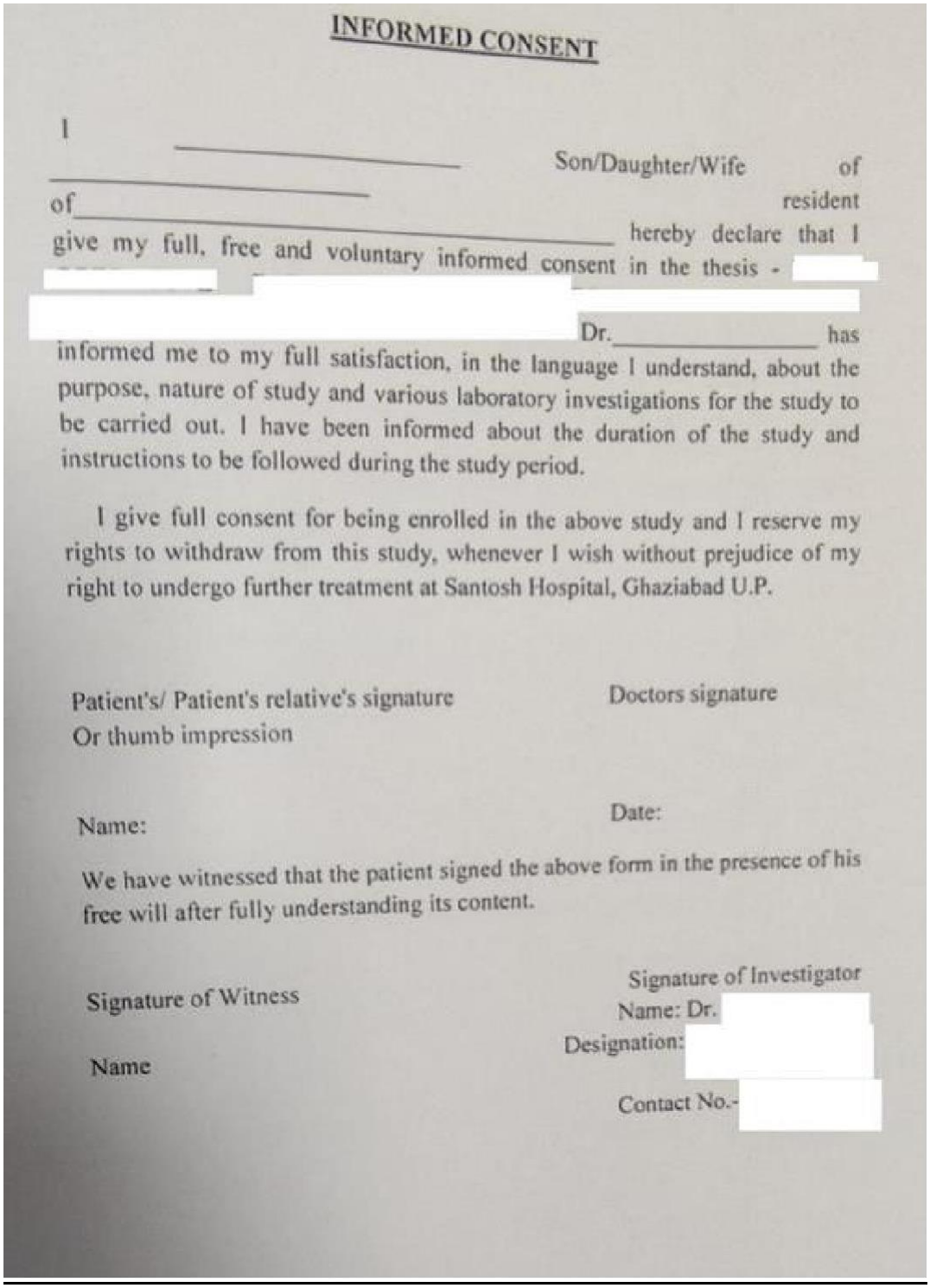
INFORMED CONSENT ENGISH



INFORMED CONSENT

I _____ Son/Daughter/Wife of _____ of _____ resident of _____ hereby declare that I give my full, free and voluntary informed consent in the thesis - _____ Dr._____ has informed me to my full satisfaction, in the language I understand, about the purpose, nature of study and various laboratory investigations for the study to be carried out. I have been informed about the duration of the study and instructions to be followed during the study period.

I give full consent for being enrolled in the above study and I reserve my rights to withdraw from this study, whenever I wish without prejudice of my right to undergo further treatment at Santosh Hospital, Ghaziabad U.P.

Patient's/ Patient's relative's signature Or thumb impression

Doctors signature

Name:

Date:

We have witnessed that the patient signed the above form in the presence of his free will after fully understanding its content.

Signature of Witness

Name

Signature of Investigator

Name: Dr.

Designation:

Contact No.-

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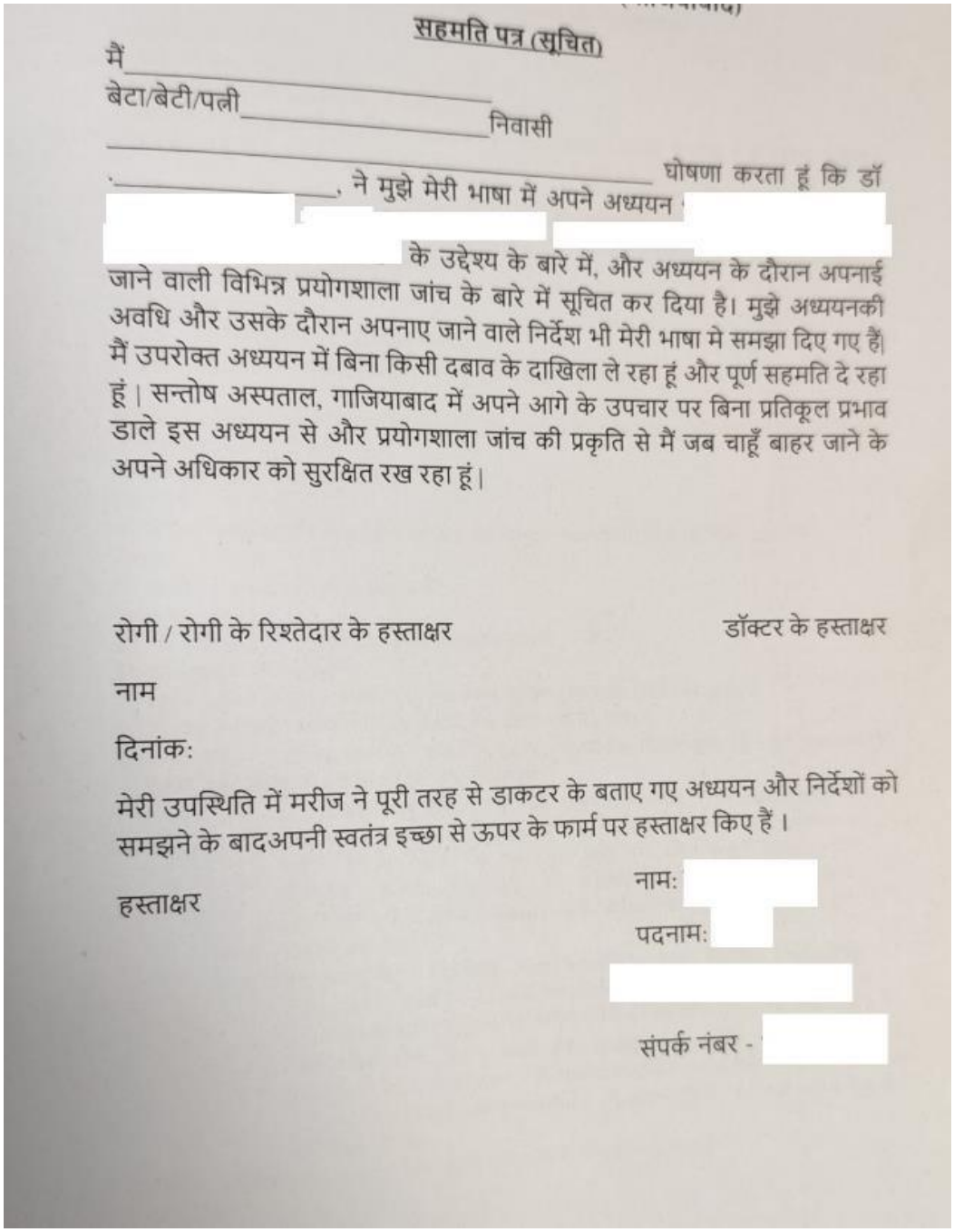

सहमति पत्र (सूचित)

मैं_____

बेटा/बेटी/पत्नी_____निवासी

_____ घोषणा करता हूं कि डॉ

._____, ने मुझे मेरी भाषा में अपने अध्ययन

के उद्देश्य के बारे में, और अध्ययन के दौरान अपनाई जाने वाली विभिन्न प्रयोगशाला जांच के बारे में सूचित कर दिया है। मुझे अध्ययनकी अवधि और उसके दौरान अपनाए जाने वाले निर्देश भी मेरी भाषा मे समझा दिए गए हैं| मैं उपरोक्त अध्ययन में बिना किसी दबाव के दाखिला ले रहा हूं और पूर्ण सहमति दे रहा हूं | सन्तोष अस्पताल, गाजियाबाद में अपने आगे के उपचार पर बिना प्रतिकूल प्रभाव डाले इस अध्ययन से और प्रयोगशाला जांच की प्रकृति से मैं जब चाहूँ बाहर जाने के अपने अधिकार को सुरक्षित रख रहा हूं|

रोगी / रोगी के रिश्तेदार के हस्ताक्षर

डॉक्टर के हस्ताक्षर

नाम

दिनांक:

मेरी उपस्थिति में मरीज ने पूरी तरह से डाकटर के बताए गए अध्ययन और निर्देशों को समझने के बादअपनी स्वतंत्र इच्छा से ऊपर के फार्म पर हस्ताक्षर किए हैं ।

हस्ताक्षर

नाम:

पदनाम:

संपर्क नंबर -

DETAILS OF PATIENT INFORMATION SHEET

HUMAN RESEARCH PROPOSAL

1. Identifying Data

   Name of Researcher: AIMAN ABBAS JAFRI

   Department: BIOCHEMISTRY

   Title of Project: “Testosterone in the development of CVD in Type 2 diabetes”

2. Description of Human Subjects Safeguards and Rights:

a) Possible psychological of physical risk of discomfort, involved in research If

   Yes/No (if no, skip time B)- No

   If yes, answer the following

   Why necessary?

1. To have the common disease
2. Specificity and sensitivity of the treatment

b) Make sure the following questions are answered in your research proposal on collection of Data:

1) When will it take place?

2) What length of time needed for complete examination of the patient?

3) What period of the time needed?

4) Which specific place within institution?

5) How much population required including number of subjects?

6) Name of instrument to be used for data collection?

7) Methodology to be used (write details)?

c) Will subjects be given research results if they so request?

Yes / No

LETTER OF ETHICAL CLEARANCE FOR DISSERTATION

FROM REGISTRAR OFFICE

SANTOSH
Deemed to be University
(Established u/s 3 of the UGC Act, 1956)

F. No. SU/R/2024/1344[3] Dated: 17.05.2024

Subject: Grant of Ethical Clearance for **Dissertation Protocol** Topic **"Testosterone in the Development of CVD in Type 2 Diabetes"**, submitted by **AIMAN ABBAS JAFRI,** student of M.Sc. Medical BIOCHEMISTRY, 2021 Batch.

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With reference to the request for grant of Ethical Clearance for the Dissertation Protocol entitled **"Testosterone in the Development of CVD in Type 2 Diabetes"**, **AIMAN ABBAS JAFRI** is informed that the **Dissertation Protocol** submitted by him/her was considered by the **Screening Committee** of the **Allied Health Sciences** in its meetings held on 07.02.2024 and 29.02.2024. The recommendations of the **Screening Committee** were considered in detail by the **Institutional Ethics Committee** in its meeting held on **15.05.2024** and the same was **approved** by the **Ethics Committee** of the **Santosh Deemed to be University, Ghaziabad, Delhi NCR**.

He /She is informed accordingly for further necessary action.

17/5/24

[Dr. EZHILRAJAN. K]
REGISTRAR

AIMAN ABBAS JAFRI
M.Sc. Medical BIOCHEMISTRY.

Copy to:

1. The Vice Chancellor
2. The Dean – Academics
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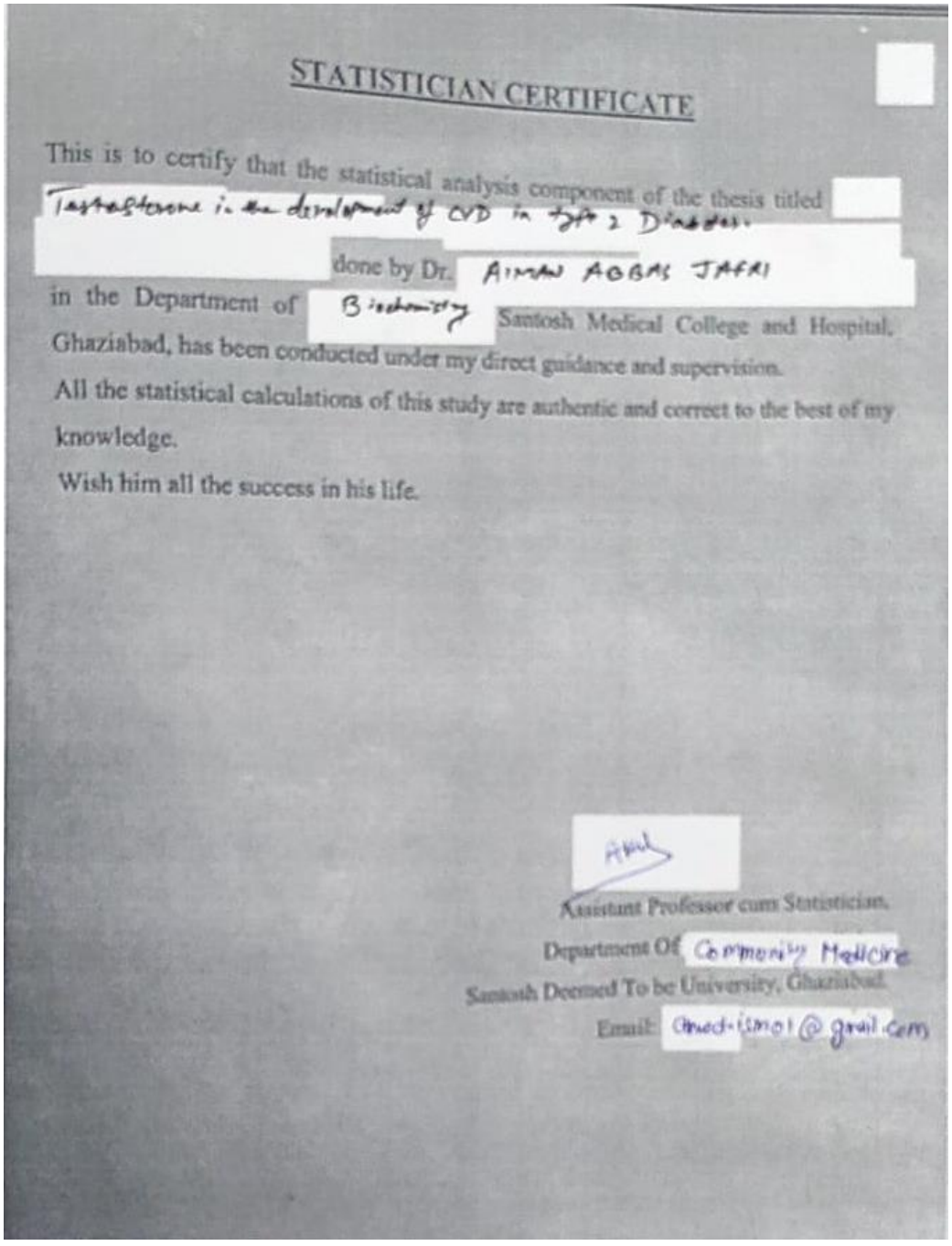
STASTICIAN CERTIFICATE



STATISTICIAN CERTIFICATE

This is to certify that the statistical analysis component of the thesis titled Testosterone in the development of CVD in type 2 Diabetes.

done by Dr. AIMAN ABBAS JAFRI

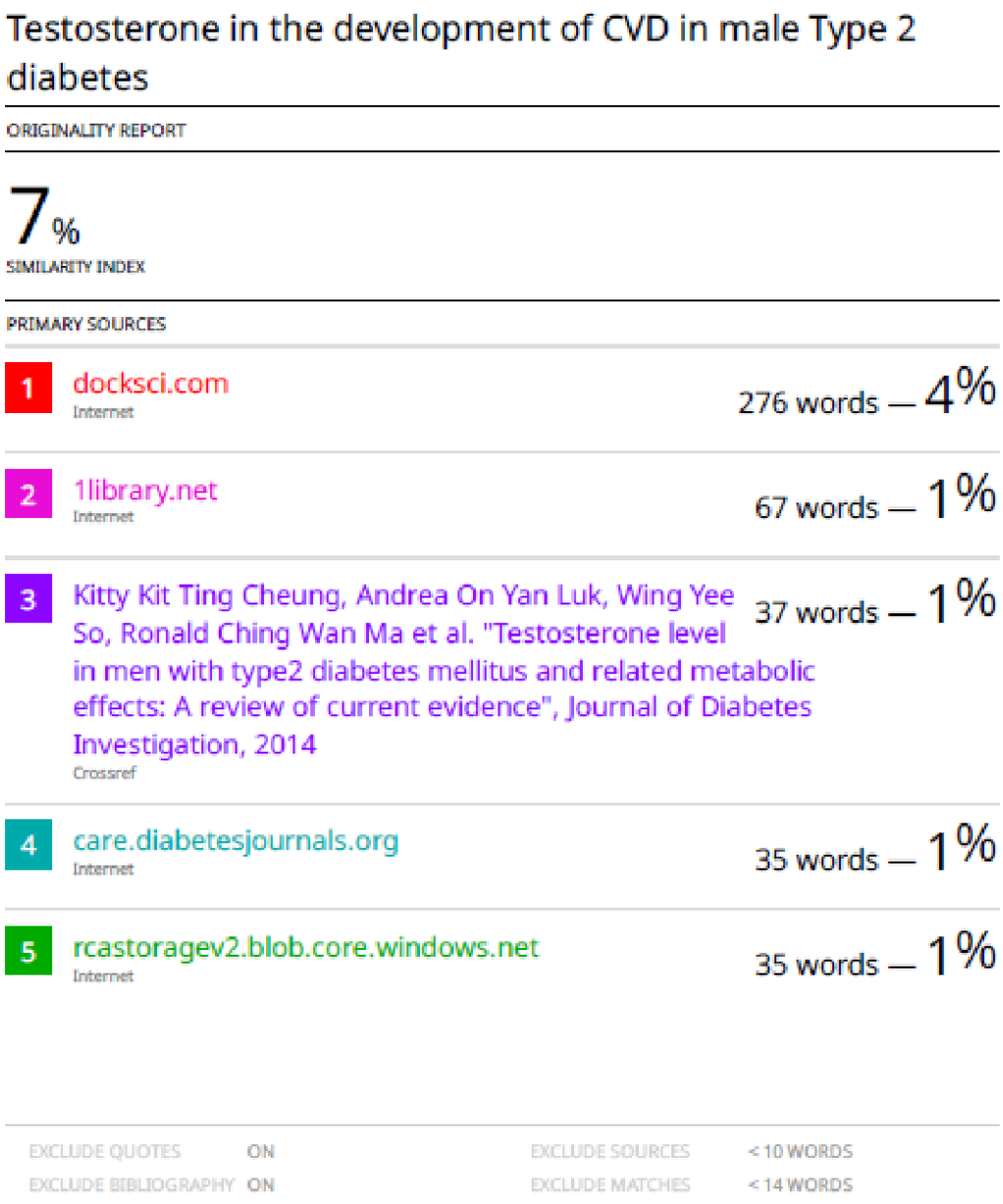
in the Department of Biochemistry Santosh Medical College and Hospital, Ghaziabad, has been conducted under my direct guidance and supervision.

All the statistical calculations of this study are authentic and correct to the best of my knowledge.

Wish him all the success in his life.

Assistant Professor cum Statistician,
Department Of Community Medicine
Santosh Deemed To be University, Ghaziabad.
Email: drmedismo1@gmail.com

PLAGIARISM CERTIFICATE



Testosterone in the development of CVD in male Type 2 diabetes

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